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STUDIES ON THE CONSTITUENTS OF ASCLEPIADACEAE PLANT—LVII[1]

THE STRUCTURES OF SIX GLYCOSIDES, WILFOSIDE C1N, C2N, C3N, C1G, C2G AND C3G, WITH NOVEL SUGAR CHAIN CONTAINING A PAIR OF OPTICALLY ISOMERIC SUGARS

SACHIKO TSUKAMOTO, KOJI HAYASHI and HIROSHI MITSUHASHI*
Faculty of Pharmaceutical Sciences, Hokkaido University,
Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060, Japan

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Abstract—Six new glycosides named wilfoside C3N (**1**), C1N (**2**), C2N (**3**), C3G (**4**), C1G (**5**) and C2G (**6**) were isolated from *Cynanchum wilfordi* HEMSLEY (Asclepiadaceae) and their structures were deduced on the basis of the chemical and spectral evidence. It is quite unusual that **2**, **3**, **5** and **6** include both D-cymarose and L-cymarose in each sugar chain.

The dried root of *Cynanchum wilfordi* HEMSLEY (Asclepiadaceae) has been used as a substitute of the tonic, crude drug Ka-shu-uh which originates from the Polygonaceous plant in Korea. In 1975, we reported the isolations of C/D *cis* polyoxypregnane esters such as caudatin (**7**), kidjoranine, penupogenin, aglycone-D, and aglycone-E from the acidic hydrolysate of the crude glycoside of this plant.[2]

In this paper we wish to describe the isolation and the structure determination of six new glycosides named wilfoside C3N (**1**), C1N (**2**), C2N (**3**), C3G (**4**), C1G (**5**) and C2G (**6**) (Scheme 1). They showed positive Liebermann–Burchard and Keller–Kiliani[3] reactions, which indicate the presence of steroidal glycosides with 2-deoxysugars. It is remarkable that the cymarose from the hydrolysate of the crude glycoside showed the specific rotation: $[\alpha]_D + 24.5°$ ($c = 1.34$, H_2O), which suggests both D-cymarose and L-cymarose are present in the ratio 2:1. The separating procedure is shown in Scheme 2.

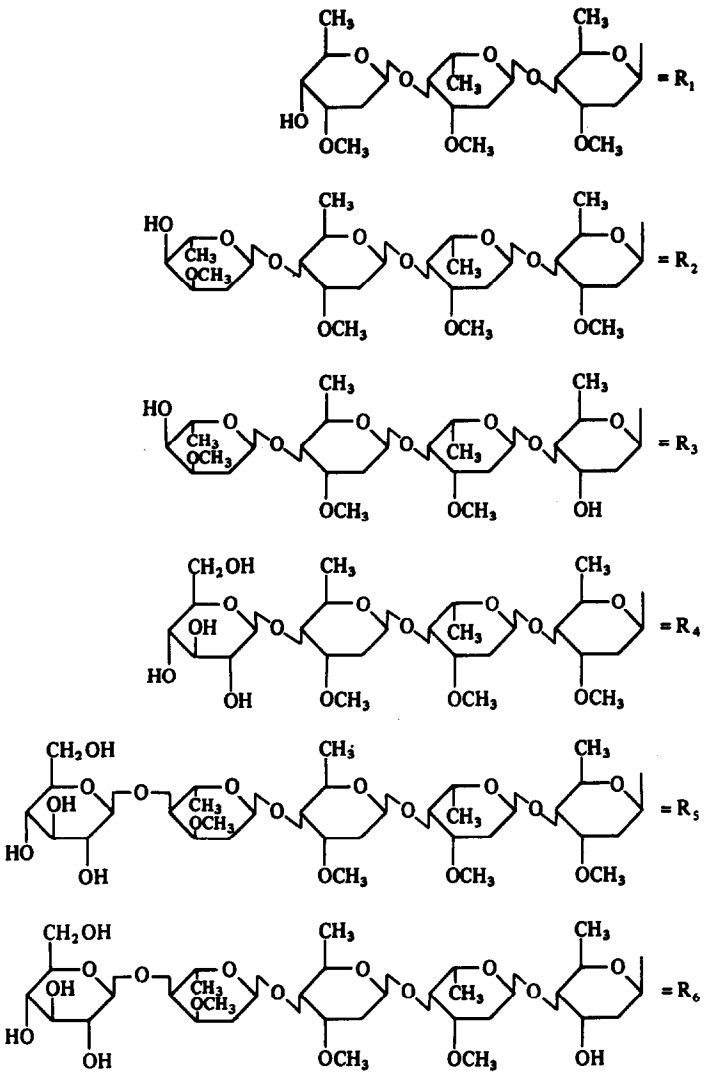
Wilfoside C3N (**1**) *and C3G* (**4**)

The 500 MHz proton magnetic resonance (^{1}H-NMR) spectrum of **1** showed the methyl signals of the aglycone moiety at δ 1.06 (6H, d, J = 6.7 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.40 (3H, s, 19-CH_3), 2.13 (3H, d, J = 1.2 Hz, 7′-CH_3), and 2.17 (3H, s, 21-CH_3), two olefinic proton signals at δ 5.37 (1H, br s, 6-CH) and 5.52 (1H, br s, 2′-CH), and the methoxyl methyl signals of sugars at δ 3.416, 3.425 and 3.427 (each 3H, s). One α-linkage and two β-linkages of sugars were revealed by the coupling constants of the anomeric proton signals at δ 4.69 (1H, dd, J = 10, 2 Hz), 4.84 (1H, dd, J = 9, 2 Hz), and 4.99 (1H, dd, J = 3.4, 1 Hz). The mild acidic hydrolysis of **1** afforded **7**, L-diginose (**8**), and cymarose, which were identified by TLC comparison with the samples obtained from the crude glycoside (Table 1). **7** and D-cymarose, $[\alpha]_D + 58.0°$ ($c = 0.30$, H_2O), were separated, and the former was confirmed by the ^{1}H-NMR spectrum. The ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** (Tables 2 and 3) also indicated the presence of **7** as the aglycone moiety and three monosaccharides, one α-L-diginopyranose and two β-D-cymaropyranoses. The chemical shifts of sugars were compared with those of the methyl glycosides (Table 4). The glycosidation shifts[4] of the aglycone carbon signals were observed at C-2 (−2.1 ppm), C-3 (+6.1 ppm) and C-4 (−4.3 ppm), therefore the sugar moiety is linked to the C-3 hydroxyl group of **7**. The chemical and spectral data on the aglycone moieties of glycosides **2**, **3**, **4**, **5** and **6** were almost the same as those of **1** (Tables 2 and 3 and Scheme 1). Therefore, these glycosides consist of **7** with sugar linkage at its C-3 hydroxyl group. The sugar sequence of **1** was deduced by the ^{13}C-NMR spectrum with partially relaxed Fourier transform (PRFT) measurements[5] (Table 3). The spectra were measured at eight pulse intervals (100, 120, 140, 150, 160, 170, 180 and 200 MS) between 180° and 90° pulses. Methine carbon signals of the terminal β-D-cymaropyranosyl and α-L-

Table 1. Acidic hydrolysis products of **1**–**6** and optical rotation of cymarose†

Glycoside	Products			$[\alpha]_D$ (cymarose)
1		D-cymarose × 2	**8** **7**	+58.0°
2	L-cymarose	D-cymarose × 2	**8** **7**	+25.9°
3	**11** L-cymarose	D-cymarose	**8** **7**	+0.5°
4	**13**	D-cymarose	**8** **7**	‡
5	**12**	D-cymarose × 2	**8** **7**	+55.3°
6	**12** **11**	D-cymarose	**8** **7**	‡

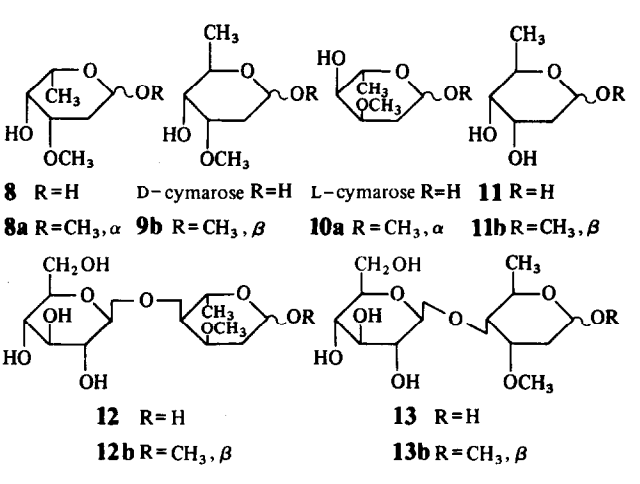
† Measured in $CHCl_3$
‡ Not measured.

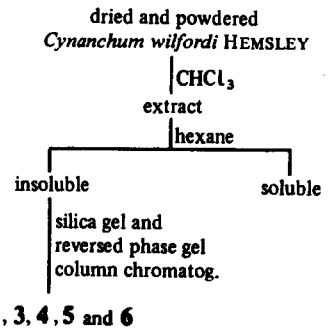


Scheme 1.

diginopyranosyl signals were recovered at 180 and 160 MS, respectively. Hence these sugar signals were distinguished from others. Thus, the structure of **1** is established as caudatin 3-*O*-*β*-D-cymaropyranosyl-(1 → 4)-*α*-L-diginopyranosyl-(1 → 4)-*β*-D-cymaropyranoside.

The carbon chemical shifts of **4** were shown in Tables 2 and 3. The terminal *β*-D-glucopyranosyl signals were confirmed by PRFT measurements. The glycosidation shifts were observed at C-3 (−0.7 ppm), C-4 (+8.9 ppm), and C-5 (−1.5 ppm) of *β*-D-cymaropyranose, to which the terminal *β*-D-glucopyranose was linked. The





Scheme 2. Isolation of glycosides.

other signals were nicely corresponded to those of **1**. The acidic hydrolysis of **4** afford **7**, **8**, cymarose, and strophanthobiose (**13**), which were identified by TLC comparison with the authentic samples (Table 1). The presence of **13** indicated that D-glucopyranose was linked to D-cymaropyranose in the molecule of **4**. The ^{1}H-NMR spectrum of **4**, similarly to **1**, showed three methoxyl methyl and three anomeric proton signals of 2-deoxysugars. Further, the anomeric proton signal of β-D-glucopyranose was revealed at δ 4.36 (1H, d, J = 7.6 Hz). Consequently, the structure of **4** is confirmed as caudatin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside.

Wilfoside C1N (**2**) *and C1G* (**5**)

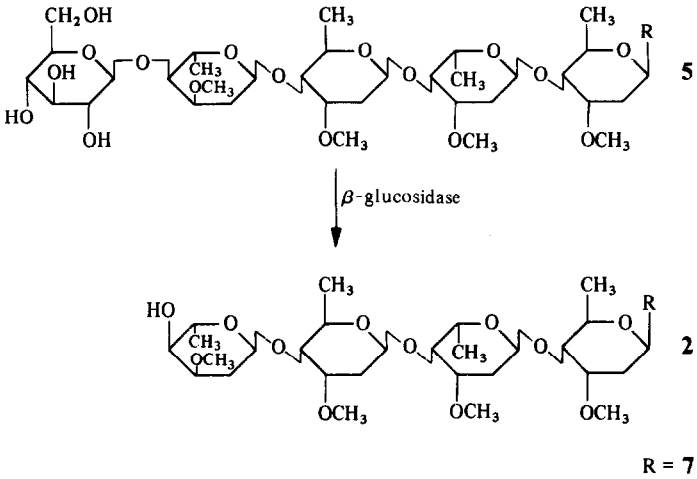
The enzymatic hydrolysis of **5** with β-glucosidase gave deglucosyl-**5** (**14**) (Scheme 3), which was identified as **2** by the ^{1}H- and ^{13}C-NMR (Tables 2 and 3) spectra. The terminal β-D-glucopyranosyl signals of **5** were confirmed by PRFT measurements. The glycosidation shifts were observed at C-3, 4 and 5 of α-L-cymaropyranose. The other signals were nicely corresponded to those of **2**. The acidic hydrolysis of **5** afforded **7**, **8**, cymarose and glaucobiose (**12**). These data indicate that D-glucopyranose was linked to L-cymaropyranose in the sugar sequence of **5**, so that the terminal sugar of **2** was deduced to be L-cymaropyranose.

The acidic hydrolysis of **2** afforded **7**, **8** and cymarose (Table 1), which were identified by TLC comparison with the authentic samples. The optical rotation of this cymarose, $[\alpha]_D$ +25.9° (c = 0.54, H_2O), proved it a mixture of D- and L-series in the ratio *ca* 2:1. The ^{1}H-NMR spectrum of **2** showed four methoxyl methyl groups. Two α-linkages and two β-linkages of four 2-deoxysugars were revealed by the coupling constants of the anomeric proton signals. In the ^{13}C-NMR spectrum of **2** the terminal α-L-cymaropyranosyl signals were easily distinguished from others by PRFT measurements (Tables 2 and 3), and on the next β-D-cymaropyranosyl signals the glycosidation shifts were Observed at C-3, 4 and 5. The rest exactly corresponded to **1**. Therefore, the structures of **2** and **5** are established as caudatin 3-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1

Table 2. ^{13}C-NMR chemical shifts[a] for **7** and aglycone moieties of **1**–**6** and **13** (δ in ppm)

	7[b]	1		4	2	5	14	3	6
C-1	39.2	39.2		39.3	39.2	39.3	39.4	39.2	39.1
2	31.9	29.8	(−2.1)	29.8	29.8	29.8	29.9	29.8	29.9
3	71.5	77.6[c]	(+6.1)	77.6[c]	77.6[c]	77.7[c]	77.6[c]	77.6[c]	77.6[c]
4	43.2	38.9	(−4.3)	39.0	38.9	39.0	39.0	38.9	39.0
5	140.5	139.3		139.4	139.3	139.3	139.4	139.3	139.4
6	118.4	119.0		119.1	119.1	119.1	119.1	119.1	119.1
7	33.8[c]	33.8[d]		33.8[d]	33.8[d]	33.8[d]	33.9[d]	33.8[d]	33.8[d]
8	74.3	74.2		74.3	74.2	74.3	74.3	74.2	74.3
9	44.5	44.6		44.6	44.5	44.6	44.6	44.6	44.6
10	37.3	37.3		37.4	37.3	37.4	37.4	37.3	37.4
11	25.0	25.0		25.0	25.0	25.0	25.0	25.0	25.1
12	72.5	72.5		72.6	72.5	72.5	72.6	72.5	72.5
13	57.9	57.9		57.9	57.9	57.9	58.0	57.9	57.9
14	89.4	89.4		89.4	89.4	89.4	89.4	89.4	89.4
15	34.7[c]	34.8[d]		34.8[d]	34.8[d]	34.8[d]	34.9[d]	34.6[d]	34.8[d]
16	32.9[c]	32.8[d]		32.9[d]	32.9[d]	32.9[d]	32.9[d]	32.8[d]	32.9[d]
17	92.3	92.3		92.3	92.3	92.3	92.4	92.3	92.4
18	10.7	10.8		10.6	10.6	10.6	10.7	10.6	10.7
19	18.3	18.4[e]		18.5[e]	18.4[e]	18.5[e]	18.2[e]	18.3[e]	18.4[e]
20	209.2	209.1		209.2	209.2	209.2	209.3	209.2	209.3
21	27.5	27.4		27.4	27.5	27.5	27.5	27.4	27.5
1′	165.9	165.8		165.9	165.8	165.9	165.9	165.9	165.9
2′	114.1	114.1		114.1	114.1	114.1	114.2	114.1	114.1
3′	165.2	165.2		165.3	165.3	165.3	165.3	165.3	165.3
4′	38.1	38.1		38.1	38.1	38.1	38.2	38.0	38.1
5′	20.8[d]	20.8		20.8[f]	20.8[f]	20.8[f]	20.9[f]	20.8[f]	20.8[f]
6′	20.9[d]	20.8		20.9[f]	20.9[f]	20.9[f]	21.0[f]	20.9[f]	20.9[f]
7′	16.4	16.4		16.4	16.4	16.5	16.5	16.4	16.4

[a] Measured in C_5D_5N with TMS as an internal standard.
[b] Assigned on the basis of off-resonance (OFR) and insensitive nuclei enhanced by population transfer (INEPT) spectra.
[c–m] Assignments in each column may be interchangeable (Tables 2 and 3).
Glycosidation shifts are given in parentheses.



Scheme 3. Enzymatic hydrolysis of **5** with β-glucosidase.

→ 4)-β-D-cymaropyranoside and caudatin 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside, respectively.

Wilfoside C2N (**3**) *and C2G* (**6**)

The acidic hydrolysis of **3** afforded **7**, **8**, cymarose and D-digitoxose (**11**), which were identified by TLC comparison with the authentic samples (Table 1). The cymarose separated from **3** gives the optical rotation, $[\alpha]_D$ +0.5° (c = 0.79, H_2O), which proved it a mixture of D- and L-series in the ratio 1 : 1. From the ^{13}C-NMR spectra of **1** and **3** (Tables 2 and 3), the structure of **3** corresponded to one replaced the inner D-cymaropyranose of **1** with D-digitoxopyranose. The PRFT measurements of **3** indicated the terminal α-L-cymaropyranosyl signals. The glycosidic linkage at C-4 of **11** in the sugar sequence of **3** was suggested by the ^{1}H-NMR spectrum of diacetate (**15**) of **3**. Owing to acetylation, 3-CH of **11** was shifted from δ 3.59 to 5.30 (1H, ddd, J = 3.1, 2.9, 2.9 Hz) and 4-CH of the terminal L-cymaropyranose from δ 3.26 to 4.68 (1H, dd, J = 8.2, 3.4 Hz), therefore, the C-3 hydroxyl group of **11** is free in this sugar sequence. The ^{1}H-NMR spectrum of **3** showed three methoxyl methyl and four anomeric proton signals of 2-deoxysugars. Thus, the structure of **3** is established as caudatin 3-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-digitoxopyranoside.

The ^{13}C-NMR spectrum of **6** was shown in Tables 2 and 3. The terminal β-D-glucopyranosyl signals of **6** was confirmed by PRFT measurements. The glycosidation shifts were observed at C-3, 4 and 5 of α-L-cymaropyranose, and the other signals were agreed with those of **3**. The acidic hydrolysis of **6** gave **7**, **8**, cymarose, **11** and **12**, which were identified by TLC comparison. The ^{1}H-NMR spectrum of **6** revealed three methoxyl methyl and five anomeric proton signals of sugars. The structure of **6** is concluded to be caudatin 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-α-L-diginopyranosyl-(1 → 4)-β-D-digitoxopyranoside.

The structures of **1**, **2** and **3** are corresponded to deglucosyl-**4**, **5** and **6**, respectively. **2** is agreed with **1** linked by α-L-cymaropyranose at the terminal site, and **3** is equivalent to one replaced the inner D-cymaropyranose of **1** with D-digitoxopyranose (Scheme 1). It is evident that these glycosides are related to each other by their biosynthetic point of view. Among the Asclepiadaceous plants, ex. *Cynanchum caudatum*[8] and *Dregea volubilis*[1] etc. contain D-cymarose. On the other hand, *C. glaucescens*[9] has L-cymarose in their glycosides. In this investigation, *C. wilfordi* comes to have both D- and L-cymarose. These facts are very interesting with respect to the chemotaxonomy among the Asclepiadaceous plants, especially in the genous *Cynanchum*. Probably, Glycosides **2**, **3**, **5** and **6** are the first known examples that contain a pair of optically isomeric sugars in each molecule.

EXPERIMENTAL

M.ps were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ with a JASCO DIP-4 digital polarimeter at room temp. UV spectra were obtained in EtOH with a Shimadzu UV-220 spectrometer, and absorption maxima are given in nm. IR spectra were recorded in $CHCl_3$ on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on JEOL FX-500 (500 MHz), FX-200 (200 MHz), or FX-100 (100 MHz) spectrometers in $CDCl_3$, $CDCl_3$–CD_3OD (1 : 1) or C_5D_5N, and ^{13}C-NMR spectra on JEOL FX-200 (50 MHz) or FX-100 (25 MHz) machine in C_5D_5N with tetramethylsilane as an internal standard. Electron impact mass spectrometry (EI-MS) was determined with a JEOL LMS-D-300 mass spectrometer and field desorption mass spectrometry (FD-MS) with a JEOL JMS-01SG-2. Thin layer chromatography (TLC) was performed on Merck precorted plates (Kiesel gel 60 F_{254}) with the following solvent systems: R_{f_1} MeOH–$CHCl_3$ (5 : 95 (v/v)), R_{f_2} MeOH–$CHCl_3$ (1 : 9), R_{f_3} MeOH–$CHCl_3$ (15 : 85), R_{f_4} H_2O–MeOH–$CHCl_3$ (1 : 3 : 12, lower layer), R_{f_5} H_2O–MeOH–$CHCl_3$ (1 : 3 : 9, lower layer), R_{f_6} acetone–hexane (1 : 1), R_{f_7} MeOH–acetone–benzene (1 : 2 : 2), and R_{f_8} hexane–ethyl acetate (1 : 3). Column chromatography was carried out on Wakogel C-300 (300 mesh), Wakogel C-200 (200 mesh), Wakogel C-100 (100 mesh), or Lobar column Lichroprep RP-8 (reversed phase).

Extraction and isolation. The dried root of *Cynanchum*

Table 3. ^{13}C-NMR chemical shifts[a] of sugar moieties of **1–6** and **14** (δ in ppm)

	1	**4**	**2**	**5**	**14**	**3**	**6**
	D-cym	D-cym	D-cym	D-cym	D-cym	digito	digito
C-1	96.1	96.1	96.1	96.1	96.1	96.3	96.4
2	35.3	35.3[g]	35.2	35.3[g]	35.3	39.8	39.7
3	77.5[c]	77.5[c]	77.4[c]	77.5[h]	77.7[c]	68.6[g]	68.7[g]
4	82.3	82.3	82.3	82.3[i]	82.3	82.5	82.6
5	69.2	69.3[h]	69.2	69.2[j]	69.2	67.9[g]	67.9[g]
6	18.7[e]	18.7[e]	18.7[e]	18.7[e]	18.8[e]	18.6[e]	18.7[e]
3-OCH_3	57.2[f]	57.2[i]	57.2[g]	57.2[k]	57.3[g]		
	dig[C]	dig	dig[B]	dig	dig	dig[B]	dig
C-1	100.8	100.8	100.8	100.9	100.9	100.7	100.7
2	32.5	32.4	32.4	32.4	32.5	32.4	32.4
3	73.9[g]	73.6[j]	73.7[h]	73.9[l]	73.9[h]	73.9[h]	74.0[h]
4	74.6[g]	74.6[j]	74.6[h]	74.6[l]	74.7[h]	74.3[h]	74.4[h]
5	67.5[g]	67.5[j]	67.4[h]	67.5[l]	67.5[h]	67.5[h]	67.5[h]
6	17.8	17.8	17.8	17.8	17.9	17.8	17.8
3-OCH_3	55.3	55.4	55.3	55.3	55.4	55.1	55.2
	D-cym[D]	D-cym	D-cym[D]	D-cym	D-cym	D-cym[C]	D-cym
C-1	99.4	99.2	99.3	99.4	99.4	99.3	99.4
2	35.3	36.1[g]	36.3	36.2[g]	36.4	36.2	36.3
3	78.8	78.1	77.7	77.7[h]	77.8[c]	77.7[c]	77.7[c]
4	74.1	83.0	82.3	82.4[i]	82.3	82.3	82.4
5	71.0	69.5[h]	69.3	69.3[j]	69.3	69.3	69.3
6	18.1[e]	18.2[e]	18.6[e]	18.5[e]	18.6[e]	18.6[e]	18.5[e]
3-OCH_3	57.9[f]	58.5[i]	58.2[g]	58.3[k]	58.3[g]	58.2	58.3
		glu[A]	L-cym[E]	L-cym	L-cym	L-cym[E]	L-cym
C-1		106.4	98.9	98.9	99.0	98.9	98.9
2		75.3	32.1	32.2	32.2	32.1	32.2
3		78.3	76.3	73.3	76.4	76.4	73.3
4		71.8	73.2	78.8	73.3	73.2	78.9
5		78.3	66.3	65.2	66.3	66.3	65.3
6		63.0	18.1[e]	18.2[e]	18.2[e]	18.1[e]	18.2[e]
3-OCH_3			56.5	56.7	56.6	56.5	56.7
				glu[A]			glu[A]
C-1				102.2			102.3
2				75.2			75.3
3				78.5			78.4[i]
4				71.7			71.8
5				78.5			78.6[i]
6				62.9			63.0

[a] Measured in C_5D_5N with TMS as an internal standard.
[c–l] Each column may be interchangeable (Tables 2 and 3).
[A–E] Methine carbon signals of their sugars were recovered at following times by PRFT measurements. [A]120 MS; [B]140 MS; [C]160 MS: [D]180 MS; [E]200 MS.
L-cym: α-L-cymaropyranosyl, D-cym: β-D-cymaropyranosyl,
dig: α-L-diginopyranosyl, digito: β-D-digitoxopyranosyl,
glu: β-D-glucopyranosyl.

wilfordi (5.2 kg), obtained from Korea, was pulverised and extracted with $CHCl_3$ at room temp. A dark brown tar (298 g) given by concentration of the extract was dissolved in $CHCl_3$ (300 ml) again and the soln was poured into hexane (2000 ml). The insoluble portion corresponded to a crude glycoside (220.3 g), which showed positive Liebermann–Burchard and Keller–Kiliani reactions. The crude glycoside (137.7 g) was subjected to column chromatography on silica gel using solvents of increasing polarity from $CHCl_3$ to MeOH–$CHCl_3$ [2:8 (v/v)] to separate fraction A (47.27 g, a crude fraction of **1**, **2** and **3**), fraction B (39.19 g, a crude fraction of **4**, **5** and **6**), and fraction C (28.50 g). Fraction A (37.50 g) was rechromatographed on silica gel using solvents of increasing polarity from MeOH–$CHCl_3$ (2:98) to MeOH–$CHCl_3$ (4:96) to separate fraction A2 (7.10 g, a crude fraction of **1** and **2**) and fraction A3 (21.59 g, a crude fraction of **3**). Fraction A2 (7.10 g) and A3 (21.59 g) were rechromatographed on silica gel using MeOH–$CHCl_3$ (1.5:98.5) and on reversed phase gel using H_2O–MeOH (15:85) to afford **1** (128.0 mg), **2** (390.3 mg) and **3** (243.0 mg). Fraction B (20.89 g) was rechromatographed on silica gel using solvents of increasing polarity from MeOH–$CHCl_3$ (7.5:92.5) to MeOH–$CHCl_3$ (2:8) to separate fraction B2 (16.10 g, a crude fraction of **5**) and fraction B4 (4.16 g, a crude fraction of **4** and **6**). Fraction B2 (5.32 g) was rechromatographed on silica gel using MeOH–$CHCl_3$ (6:94) and on reversed phase gel using H_2O–MeOH (2:8) to afford **5** (445.9 mg). Fraction B4 (2.91 g) was rechromatographed on silica gel using each of MeOH–$CHCl_3$ (8:92) and hexane–acetone (1:3), and on reversed phase gel using H_2O–MeOH (15:85) to afford **4** (72.0 mg) and **6** (56.9 mg).

Sugar obtained from the crude glycosides. The crude sugar fraction (61.50 g) obtained by the acidic hydrolysis of the crude glycoside[2] was chromatographed on silica gel using solvents of increasing polarity from MeOH–$CHCl_3$ (2:8) to separate fraction S1 (26.32 g, a crude fraction of methyl glycosides of cymarose and **8**), fraction S2 (4.51 g, a crude fraction of **11b**), and fraction S3 (12.99 g, a crude fraction of **12b**).

A soln of fraction S1 (12.60 g) in dioxane (120 ml) was

Table 4. ^{13}C-NMR chemical shifts[a] of **8a**, **9b**, **10a**, **11b**, **12b** and **13b** (δ in ppm)

	8a	**9b**[b]	**10a**[b]	**11b**[c]	**12b**[c]	**13b**[b]
C-1	99.2	99.4	97.6	99.6	99.5	99.3
2	30.4	35.1	31.9	39.1	35.3	36.1
3	75.9[d]	78.5	76.5	68.3	74.6	77.8
4	67.6[d]	74.0	73.2	74.0	79.2	83.2
5	66.8[d]	71.0	65.2	70.3	69.6	69.4
6	17.5	18.9	18.5	18.8	19.0	18.6
OCH_3	54.5	56.0	54.7	55.9	55.9	56.0
	55.0	57.8	56.7		58.1	58.4
C-1′					102.2	106.5
2′					75.2	75.4
3′					78.6	78.4
4′					71.9	71.9
5′					78.6	78.4
6′					63.0	63.1

[a] Measured in C_5D_5N with TMS as an internal standard.
[b] See Ref. 7.
[c] Assigned by selective decoupling experiments.
[d] May be interchangeable.

allowed to react with 0.2 N H_2SO_4 (40 ml) at 90° for 30 min, and neutralized with satd $Ba(OH)_2$. The products were chromatographed on silica gel using H_2O–MeOH–$CHCl_3$ (1:3:18, lower layer) to separate cymarose (1.504 g) and **8** (459.0 mg). Syrup of **8** was crystallized by acetone–hexane to afford 373.8 mg of **8**, m.p. 89–92°, $[\alpha]_D$ −60.6° (c = 0.94, H_2O). (Found: C, 51.83; H, 8.77. Calc for $C_7H_{14}O_4$: C, 51.84; H, 8.70%.) FD-MS m/z: 162 (M^+). A soln of **8** (273.8 mg) in MeOH (2 ml) was allowed to react with 1% H_2SO_4–MeOH (2 ml) at room temp for 20 min, then H_2O (2 ml) was added to this and the mixture was neutralized with satd $Ba(OH)_2$. The ppts were filtered off, and the filtrate was evaporated to give **8** (275.5 mg). The products were chromatographed on silica gel using EtOAc–hexane (2:5) to afford **8c** (50.2 mg), **8a** (74.1 mg), and a mixture (120.8 mg) of **8d** and **8b**. **8d** and **8b** were separated by silica gel column chromatography using MeOH–$CHCl_3$ (1:9) to give **8d** (88.2 mg) and **8b** (16.5 mg). **8a**: R_{f_1} 0.66 and R_{f_8} 0.36; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.31 (3H, d, J = 6.7 Hz, 6-CH_3), 1.84 (1H, ddd, J = 13.1, 11.6, 3.4 Hz, 2-CH_{ax}), 1.90 (1H, ddd, J = 13.1, 5.5, 1.0 Hz, 2-CH_{eq}), 3.33, 3.39 (each 3H, s, 1,3-OCH_3), 3.62 (1H, ddd, J = 11.6, 5.5, 3.1 Hz, 3-CH), 3.78 (1H, dd, J = 3.1, 2 Hz, 4-CH), 3.83 (1H, dq, J = 6.7, 2 Hz, 5-CH), 4.82 (1H, dd, J = 3.4, 1.0 Hz, 1-CH). ^{13}C-NMR (Table 4). **8b**: R_{f_1} 0.56 and R_{f_8} 0.33; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.37 (3H, d, J = 6.4 Hz, 6-CH_3), 1.67 (1H, ddd, J = 12.5, 11.9, 9.8 Hz, 2-CH_{ax}), 2.02 (1H, ddd, J = 12.5, 4.9, 2.1 Hz, 2-CH_{eq}), 3.35 (1H, ddd, J = 11.9, 4.9, 3.1 Hz, 3-CH), 3.40 (3H, s, OCH_3), 3.46 (1H, dq, J = 6.4, 2 Hz, 5-CH), 3.50 (3H, s, OCH_3), 3.71 (1H, dd, J = 3.1, 2 Hz, 4-CH), 4.32 (1H, dd, J = 9.8, 2.1 Hz, 1-CH). ^{13}C-NMR (50 MHz, C_5D_5N): δ 17.5 (C-6), 32.5 (C-2), 55.3, 55.9 (1, 3-OCH_3), 67.0, 71.4, 79.0 (C-3, 4, 5), 101.8 (C-1). **8c**: R_{f_1} 0.87 and R_{f_8} 0.44, ^{1}H-NMR (100 MHz, $CDCl_3$): δ 1.23 (3H, d, J = 6.4 Hz, 6-CH_3), 2.1–2.3 (2H), 3.30, 3.41 (each 3H, s, 1,3-OCH_3), 3.6–3.8 (1H, m), 3.9–4.1 (2H), 5.12 (1H, dd, J = 5.2, 2.6 Hz, 1-CH). **8d**: R_{f_1} 0.70 and R_{f_8} 0.33, ^{1}H-NMR (100 MHz, $CDCl_3$): δ 1.27 (3H, d, J = 6.1 Hz, 6-CH_3), 2.0–2.2 (2H), 3.34, 3.39 (each 3H, s, 1,3-OCH_3), 3.6–3.9 (3H), 5.06 (1H, dd, J = 4.4, 2.0 Hz, 1-CH).

Syrup of cymarose crystallized by drying under vacuum to give needles, m.p. 86–87°, $[\alpha]_D$ +24.5° (c = 1.34, H_2O). (Found: C, 51.55; H, 8.73. Calc for $C_7H_{14}O_4$: C, 51.84; H, 8.70%.) EI-MS m/z: 162 (M^+).

Fraction S2 (2.62 g) was rechromatographed on silica gel using acetone–hexane (1:2) to give **11b** (182.0 mg). ^{1}H-NMR (200 MHz, C_5D_5N): δ 1.58 (3H, d, J = 5.9 Hz, 6-CH_3), 1.97 (1H, ddd, J = 13.2, 9.8, 2.9 Hz, 2-CH_{ax}), 2.40 (1H, ddd, J = 13.2, 3.4, 2.0 Hz, 2-CH_{eq}), 3.53 (3H, s, 1-OCH_3), 3.60 (1H, dd, J = 9.3, 2.9 Hz, 4-CH), 4.26 (1H, dq, J = 9.3, 5.9 Hz, 5-CH), 4.42 (1H, ddd, J = 3.4, 2.9, 2.9 Hz, 3-CH), 5.12 (1H, dd, J = 9.8, 2.0 Hz, 1-CH). The carbon chemical shifts of **11b** were assigned by selective decoupling (SEL) experiments (Table 4). Irradiation of the proton signals at δ 3.60 (4-CH), 4.25 (5-CH) and 4.42 (3-CH) caused the doublet carbon signals at δ 74.0, 70.3 and 68.3 to collapse to singlets, respectively. A soln of **11b** (182.0 mg) in dioxane (2.5 ml) was allowed to react with 0.1 N H_2SO_4 (2.5 ml) at 60° for 2 hr, and the mixture was neutralized with satd $Ba(OH)_2$. The ppts were filtered off and the filtrate was evaporated. The product was purified by column chromatography to afford a syrup of **11**, which was crystallized by hexane–EtOAc to give 19.1 mg of needles, m.p. 104–107°, $[\alpha]_D$ +48.4° (c = 1.00, H_2O, FD-MS m/z: 148 (M^+).

Fraction S3 (7.53 g) was chromatographed on silica gel to give a syrup of **12b**, which was crystallized by EtOAc to afford 1.181 g of needles, m.p. 95.5–98°, $[\alpha]_D$ −33.2° (c = 0.98, MeOH). (Found: C, 48.31; H, 7.88. Calc for $C_{14}H_{26}O_9 \cdot 1/2\,H_2O$: C, 48.41; H, 7.78%.) FD-MS m/z: 339 ($M+H)^+$. The proton and carbon chemical shifts were assigned by decoupling and SEL experiments, respectively. ^{1}H-NMR (500 MHz, C_5D_5N): δ 1.48 (3H, d, J = 6.1 Hz, 6-CH_3), 1.67 (1H, ddd, J = 13.4, 9.1, 2.4 Hz, 2-CH_{ax}), 2.29 (1H, ddd, J = 13.4, 4.3, 2.1 Hz, 2-CH_{eq}), 3.45, 3.55 (each 3H, s, 1, 3-OCH_3), 3.91 (1H, dd, J = 8.9, 2.8 Hz, 4-CH), 3.96 (1H, ddd, J = 8.9, 5.5, 2.1 Hz, 5′-CH), 3.98 (1H, dd, J = 8.9, 7.6 Hz, 2′-CH), 4.00 (1H, ddd, J = 4.3, 2.8, 2.4 Hz, 3-CH), 4.20 (1H, t, J = 8.9 Hz, 4′-CH), 4.24 (1H, t, J = 8.9 Hz, 3′-CH), 4.25 (1H, dq, J = 8.9, 6.1 Hz, 5-CH), 4.36 (1H, dd, J = 11.6, 5.5 Hz, 6′-CH), 4.53 (1H, dd, J = 11.6, 2.1 Hz, 6′-CH), 4.89 (1H, dd, J = 9.1, 1.8 Hz, 1-CH), 4.98 (1H, d, J = 7.6 Hz, 1′-CH). ^{13}C-NMR (Table 4).

Wilfoside C3N (**1**). An amorphous powder, R_{f_1} 0.37 and R_{f_6} 0.46, m.p. 124–126.5°, $[\alpha]_D$ +14.8° (c = 0.96, $CHCl_3$). (Found: C, 63.53; H, 8.47. Calc for $C_{49}H_{78}O_{16}$: C, 63.75; H, 8.45%.) UV $\lambda_{max}^{ethanol}$ nm (log ε): 277 (3.85), 216 (3.86). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1705, 1640, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 6.7 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.23, 1.24, 1.31 (each 3H, d, J = 6.1 Hz, 6-CH_3 of sugar moiety), 1.40 (3H, s, 19-CH_3), 2.13 (3H, d, J = 1.2 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 2.85 (1H, m, 3-CH), 3.416, 3.425, 3.427 (each 3H, s, 3-OCH_3 of sugar moiety), 4.56 (1H, dd, J = 8.2, 7.6 Hz, 12-CH), 4.69 (1H, dd, J = 10, 2 Hz, anomeric H), 4.84 (1H, dd, J = 9, 2 Hz, anomeric H), 4.99 (1H, dd, J = 3.4, 1 Hz, anomeric H), 5.37 (1H, br s, 6-CH), 5.52 (1H, br s, 2′-CH). ^{13}C-NMR (Tables 2 and 3).

Acidic hydrolysis of **1**. A soln of **1** (58.1 mg) in MeOH (12 ml) was allowed to react with 0.2 N H_2SO_4 (4 ml) at 60° for 15 min, then H_2O (12 ml) was added to this and the whole was concentrated to 16 ml. Then, the soln was kept at 60° for a further 30 min, and extracted with ether (15 ml). The ether layer was washed with satd $NaHCO_3$ (5 ml × 2) and satd NaCl (5 ml

× 2), and the solvent was evaporated to give **7** (10.8 mg). R_{f_2} 0.33 and R_{f_6} 0.35. ^{1}H-NMR (100 MHz, $CDCl_3$): δ 1.07 (6H, d, J = 6.8 Hz, 5′, 6′-CH_3), 1.15 (3H, s, 18-CH_3), 1.41 (3H, s, 19-CH_3), 2.13 (3H, d, J = 1.0 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 4.56 (1H, br t, J = 7 Hz, 12-CH), 5.37 (1H, br s, 6-CH), 5.53 (1H, br s, 2′-CH). The aqueous layer was neutralized with satd $Ba(OH)_2$. The ppts were filtered off, and the filtrate was evaporated to give a mixture of **8** and cymarose, which were identified by TLC comparison with the authentic samples. R_f values: **8** (R_{f_4} 0.56, R_{f_6} 0.35) and cymarose (R_{f_4} 0.59, R_{f_6} 0.42). The mixture was chromatographed on silica gel using H_2O–MeOH–$CHCl_3$ (1:3:18, lower layer) to afford a syrup of cymarose (3.5 mg), $[\alpha]_D$ + 58.0° (*c* = 0.30, H_2O).

Wilfoside C1N (**2**). An amorphous powder, R_{f_1} 0.36 and R_{f_6} 0.44, m.p. 140–142.5°, $[\alpha]_D$ − 44.7° (*c* = 0.93, $CHCl_3$). (Found: C, 62.38; H, 8.56. Calc for $C_{56}H_{90}O_{19} \cdot 2/3H_2O$: C, 62.32; H, 8.53%.) UV $\lambda_{max}^{ethanol}$ nm (log ε): 221 (3.60), 281 (3.70). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1710, 1640, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 7.0 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.22, 1.23, 1.24, 1.26 (each 3H, d, J = 6.3 Hz, 6-CH_3 of sugar moiety), 1.40 (3H, s, 19-CH_3), 2.12 (3H, d, J = 1.2 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 2.85 (1H, m, 3-CH), 3.39, 3.41, 3.42, 3.47 (each 3H, s, 3-OCH_3 of sugar moiety), 4.57 (1H, dd, J = 9.2, 6.4 Hz, 12-CH), 4.77 (1H, dd, J = 10, 2 Hz, anomeric H), 4.79 (1H, dd, J = 3, 1 Hz, anomeric H), 4.83 (1H, dd, J = 9.6, 2 Hz, anomeric H), 4.98 (1H, dd, J = 3.4, 1 Hz, anomeric H), 5.37 (1H, br s, 6-CH), 5.52 (1H, br s, 2′-CH). ^{13}C-NMR (Tables 2 and 3).

Acidic hydrolysis of **2**. A solution of **2** (104.1 mg) in MeOH (30 ml) was allowed to react with 0.2 N H_2SO_4 (10 ml) at 60° for 20 min, then H_2O (30 ml) was added to this and the whole was concentrated to 40 ml. The solution was kept at 60° for a further 30 min, and extracted with ether (40 ml). The ether layer was washed with satd $NaHCO_3$ (10 ml × 2) and satd NaCl (10 ml × 2), and the solvent was evaporated to give **7** (36.2 mg). R_{f_2} 0.33 and R_{f_6} 0.35. ^{1}H-NMR (100 MHz, $CDCl_3$): δ 1.07 (6H, d, J = 6.8 Hz, 5′, 6′-CH_3), 1.15 (3H, s, 18-CH_3), 1.41 (3H, s, 19-CH_3), 2.13 (3H, d, J = 1.0 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 4.56 (1H, br t, J = 7 Hz, 12-CH), 5.37 (1H, br s, 6-CH), 5.53 (1H, br s, 2′-CH). The aqueous layer was neutralized with satd $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a mixture of **8** and cymarose, which were identified by TLC comparison with the authentic samples. R_f values: **8** (R_{f_4} 0.56, R_{f_6} 0.35) and cymarose (R_{f_4} 0.59, R_{f_6} 0.42). The mixture (56.2 mg) was chromatographed on silica gel using H_2O–MeOH–$CHCl_3$ (1:3:18, lower layer) to afford a syrup of cymarose (18.5 mg), $[\alpha]_D$ + 25.9° (*c* = 0.54, H_2O), and a syrup of diginose (6.4 mg), $[\alpha]_D$ − 60.0° (*c* = 0.60, H_2O).

Wilfoside C2N (**3**). An amorphous powder, R_{f_1} 0.27 and R_{f_6} 0.32, m.p. 142–143°, $[\alpha]_D$ − 50.3° (*c* = 1.38, $CHCl_3$). (Found: C, 61.02; H, 8.33. Calc for $C_{55}H_{88}O_{19} \cdot 3/2H_2O$: C, 61.15; H, 8.42%.) UV $\lambda_{max}^{ethanol}$ nm (log ε): 224 (3.71), 282 (3.80). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1705, 1640, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 6.1 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.24, 1.26 (each 3H, d, J = 6.1, 6.4 Hz, respectively, 6-CH_3 of sugar moiety), 1.41 (3H, s, 19-CH_3), 2.13 (3H, s, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 2.86 (1H, m, 3-CH), 3.39, 3.43, 3.47 (each 3H, s, 3-OCH_3 of sugar moiety), 4.56 (1H, t, J = 7.8 Hz, 12-CH), 4.77 (1H, dd, J = 10, 2 Hz, anomeric H), 4.79 (1H, dd, J = 3, 1 Hz, anomeric H), 4.94 (1H, dd, J = 9.3, 2 Hz, anomeric H), 5.06 (1H, dd, J = 3, 1 Hz, anomeric H), 5.36 (1H, br s, 6-CH), 5.52 (1H, br s, 2′-CH). ^{13}C-NMR (Tables 2 and 3).

Acidic hydrolysis of **3**. A soln of **3** (82.6 mg) in MeOH (15 ml) was allowed to react with 2 N H_2SO_4 (5 ml) at 60° for 15 min, then H_2O (15 ml) was added to this and the whole was concentrated to 20 ml. The soln was kept at 60° for a further 30 min, and extracted with ether (20 ml). The ether layer was washed with satd $NaHCO_3$ (10 ml × 2) and satd NaCl (10 ml × 2), and solvent was evaporated to give **7** (10.1 mg). R_{f_2} 0.33 and R_{f_6} 0.35. ^{1}H-NMR (100 MHz, $CDCl_3$): δ 1.07 (6H, d, J = 6.8 Hz, 5′, 6′-CH_3), 1.15 (3H, s, 18-CH_3), 1.41 (3H, s, 19-CH_3), 2.13 (3H, d, J = 1.0 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 4.56 (1H, br t, J = 7 Hz, 12-CH), 5.37 (1H, br s, 6-CH), 5.53 (1H, br s, 2′-CH). The aqueous layer was neutralized with satd $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a mixture of **8**, cymarose and **11**, which were identified by TLC comparison with the authentic samples. R_f values: **8** (R_{f_4} 0.56, R_{f_6} 0.35), cymarose (R_{f_4} 0.59, R_{f_6} 0.42), and **11** (R_{f_4} 0.42, R_{f_6} 0.04). The mixture (45.8 mg) was chromatographed on silica gel using H_2O–MeOH–$CHCl_3$ (1:3:18, lower layer) to afford a syrup of pure cymarose (8.1 mg), $[\alpha]_D$ + 0.5° (*c* = 0.79, H_2O).

*Acetyl-***3** (**15**). **3** (18.0 mg) was dissolved in pyridine (1 ml) and to this acetic anhydride (0.8 ml) was added. The reaction mixture was kept at room temperature overnight added H_2O (20 ml), and extracted with $CHCl_3$ (10 ml). $CHCl_3$ layer was washed with 2 N HCl (10 ml × 2), satd $NaHCO_3$ (10 ml × 2), and satd NaCl (10 ml × 2), and dried over Na_2SO_4. Then, the solvent was evaporated to give a syrup, which was purified by silica gel column chromatography using MeOH–$CHCl_3$ (2:98) to afford **15** (7.1 mg). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.07 (6H, d, J = 6.7 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.18, 1.21, 1.23, 1.24 (each 3H, d, J = 6.4 Hz, 6-CH_3 of sugar moiety), 1.41 (3H, s, 19-CH_3), 2.11, 2.12 (each 3H, s, $OCOCH_3$), 2.13 (3H, d, J = 1.2 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 3.35, 3.40, 3.48 (each 3H, s, 3-OCH_3 of sugar moiety), 3.57 (1H, m, 3-CH), 4.57 (1H, br t, J = 7.7 Hz, 12-CH), 4.68 (1H, dd, J = 8.2, 3.4 Hz, 4-CH of α-L-cymaropyranose), 4.75 (1H, dd, J = 10, 2 Hz, anomeric H), 4.81 (1H, dd, J = 3, 1 Hz, anomeric H), 4.82 (1H, dd, J = 10, 2 Hz, anomeric H), 4.98 (1H, dd, J = 3, 1 Hz, anomeric H), 5.30 (1H, ddd, J = 3.1, 2.9, 2.9 Hz, 3-CH of β-D-digitoxopyranose), 5.37 (1H, br s, 6-CH), 5.52 (1H, br s, 2′-CH).

Wilfoside C3G (**4**). An amorphous powder, R_{f_3} 0.38, m.p. 163–167°, $[\alpha]_D$ + 5.9° (*c* = 1.01, $CHCl_3$). (Found: C, 60.62; H, 8.15. Calc for $C_{55}H_{88}O_{21}$: C, 60.88; H, 8.18%.) UV $\lambda_{max}^{ethanol}$ nm (log ε): 220 (4.39), 279 (4.01). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1720, 1690, 1640, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 6.7 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.41 (3H, s, 19-CH_3), 2.12 (3H, d, J = 0.9 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 3.40 (6H, s, 3-OCH_3 × 2 of sugar moiety), 3.42 (3H, s, 3-OCH_3 of sugar moiety), 4.36 (1H, d, J = 7.6 Hz, anomeric H of β-D-glucopyranose), 4.56 (1H, br t, J = 7 Hz, 12-CH), 4.76 (1H, dd, J = 10, 2 Hz, anomeric H), 4.84 (1H, dd, J = 10, 2 Hz, anomeric H), 4.98 (1H, dd, J = 3, 1 Hz, anomeric H), 5.37 (1H, br s, 6-CH), 5.52 (1H, br s, 2′-CH). ^{13}C-NMR (Tables 2 and 3).

Acidic hydrolysis of **4**. A soln of **4** (1 mg) in MeOH (1 ml) was allowed to react with 0.1 N H_2SO_4 (1 ml) at 60° for 15 min, then H_2O (1 ml) was added to this and the whole was concentrated to 2 ml. The soln was kept at 60° for a further 30 min, and neutralized with satd $Ba(OH)_2$. The ppts were filtered off and the filtrate was evaporated to dryness. The products were identified as **7**, **8**, cymarose, and **13** by TLC comparison with the authentic samples. R_f values: **7** (R_{f_2} 0.33, R_{f_6} 0.35), **8** (R_{f_4} 0.56, R_{f_6} 0.35), cymarose (R_{f_4} 0.59, R_{f_6} 0.42), and **13** (R_{f_5} 0.17, R_{f_7} 0.31).

Wilfoside C1G (**5**). An amorphous powder, R_{f_3} 0.47, m.p. 143–147°, $[\alpha]_D$ − 31.8° (*c* = 0.98, $CHCl_3$). (Found: C, 60.39; H, 8.11. Calc for $C_{62}H_{100}O_{24}$: C, 60.57; H, 8.20%.) UV $\lambda_{max}^{ethanol}$ nm (log ε): 216 (4.50), 279 (4.19). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1705, 1640, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 7.0 Hz, 5′, 6′-CH_3), 1.13 (3H, s, 18-CH_3), 1.22, 1.24, 1.25, 1.26 (each 3H, s, J = 6.0 Hz, 6-CH_3 of sugar moiety), 1.40 (3H, s, 19-CH_3), 2.12 (3H, d, J = 0.9 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 3.38, 3.41, 3.42, 3.45 (each 3H, s, 3-OCH_3 of sugar moiety), 4.39 (1H, d, J = 7.6 Hz, anomeric H of β-D-glucopyranose), 4.56 (1H, br t, J = 7.6 Hz, 12-CH), 4.76 (1H, dd, J = 9.8, 2 Hz, anomeric H), 4.80 (1H, dd, J = 3, 1 Hz, anomeric H), 4.84 (1H, dd, J = 9.8, 2 Hz, anomeric H), 4.98 (1H, dd, J = 3, 1 Hz, anomeric H), 5.36 (1H, br s, 6-CH), 5.52 (1H, br s, 2′-CH). ^{13}C-NMR (Tables 2 and 3).

Acidic hydrolysis of **5**. A soln of **5** (81.9 mg) in MeOH (12 ml) was allowed to react with 2 N H_2SO_4 (4 ml) at 60° for 15 min, then H_2O (12 ml) was added to this and the whole was concentrated to 16 ml. The solution was kept at 60° for a further 30 min, and extracted with ether (15 ml). The ether layer was washed with satd $NaHCO_3$ (10 ml × 2) and satd NaCl (10 ml × 2), and the solvent was evaporated to give **7** (23.7 mg). R_{f_2} 0.33 and R_{f_6} 0.35. ^{1}H-NMR (100 MHz, $CDCl_3$): δ 1.07 (6H, d, J = 6.8 Hz, 5′, 6′-CH_3), 1.15 (3H, s, 18-CH_3), 1.41 (3H, s, 19-CH_3), 2.13 (3H, d, J = 1.2 Hz, 7′-CH_3), 2.17 (3H, s, 21-CH_3), 5.37 (1H, br s, 6-CH), 5.53 (1H, br s, 2′-CH). The aqueous layer

was neutralized with satd $Ba(OH)_2$. The ppts were filtered off and the filtrate was evaporated to give a mixture of **8**, cymarose and **12**, which were identified by TLC comparison with the authentic samples. R_f values: **8** (R_{f_4} 0.56, R_{f_6} 0.35), cymarose (R_{f_4} 0.59, R_{f_6} 0.42), and **12** (R_{f_5} 0.16, R_{f_7} 0.30). The mixture (41.0 mg) was chromatographed on silica gel using H_2O–MeOH–$CHCl_3$ (1:3:18, lower layer) to afford a syrup of cymarose (4.9 mg), $[\alpha]_D$ + 55.3° (c = 0.49, H_2O), and a syrup of **12** (8.4 mg), which was dissolved in 0.5% H_2SO_4–MeOH (1 ml). The solution was kept at room temp for 10 min, and H_2O (1 ml) was added to this. The mixture was neutralized with satd $Ba(OH)_2$. The ppts were filtered off and the filtrate was evaporated to afford a syrup, which was purified by silica gel column chromatography using H_2O–MeOH–$CHCl_3$ (1:3:12, lower layer) to give a pure syrup of **12b** (5.6 mg), $[\alpha]_D$ −31.4° (c = 0.51, MeOH). R_{f_5} 0.36 and R_{f_7} 0.44. ^{1}H-NMR [200 MHz, $CDCl_3$–CD_3OD (1:1)]: δ 1.31 (3H, d, J = 6.4 Hz, 6-CH_3), 1.59 (1H, ddd, J = 14.2, 8.3, 2.9 Hz, 2-CH_{ax}), 2.27 (1H, ddd, J = 14.2, 4.9, 2.0 Hz, 2-CH_{eq}), 3.31 (1H, ddd, J = 9, 4.9, 2.9 Hz, 5'-CH), 3.43, 3.47 (each 3H, s, 1, 3-OCH_3), 3.54 (1H, dd, J = 8.3, 2.9 Hz, 4-CH), 3.76 (1H, dd, J = 12.2, 4.9 Hz, 6'-CH), 3.86 (1H, dd, J = 12.2, 2.9 Hz, 6'-CH), 3.94 (1H, dq, J = 8.3, 6.4 Hz, 5-CH), 4.35 (1H, d, J = 7.3 Hz, 1'-CH), 4.67 (1H, dd, J = 8.3, 2.0 Hz, 1-CH). ^{13}C-NMR (50 MHz, C_5D_5N): δ 19.0 (C-6), 35.3 (C-2), 55.9, 58.1 (1, 3-OCH_3), 63.0 (C-6'), 69.6 (C-5), 71.9 (C-4'), 74.6 (C-3'), 75.3 (C-2'), 78.6 (C-3), 79.2 (C-4), 99.5 (C-1), 102.2 (C-1').

Enzymatic hydrolysis of **5** *with β-glucosidase.* A suspension (2 ml) of **5** (39.6 mg) in 0.3 M NaOAc buffer solution adjusted to pH 5.5 was added to a suspension (5 ml) of β-glucosidase (107.7 mg), which was prepared from a snail (*Fruticicola gainesil*), and kept at 37° for 183.5 hr. The products were extracted with $CHCl_3$ (28 ml) and the solvent was evaporated to give a syrup (24.4 mg), which was chromatographed to afford deglucosyl-**5** (**14**, 19.1 mg), $[\alpha]_D$ −40.0° (c = 0.91, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 6.7 Hz, 5', 6'-CH_3), 1.13 (3H, s, 18-CH_3), 1.22, 1.23, 1.24, 1.26 (each 3H, d, J = 6.4 Hz, 6-CH_3 of sugar moiety), 1.40 (3H, s, 19-CH_3), 2.12 (3H, d, J = 0.9 Hz, 7'-CH_3), 2.17 (3H, s, 21-CH_3), 2.86 (1H, m, 3-CH), 3.38, 3.41, 3.42, 3.47 (each 3H, s, 3-OCH_3 of sugar moiety), 4.56 (1H, dd, J = 8.6, 7.3 Hz, 12-CH), 4.77 (1H, dd, J = 9.5, 2 Hz, anomeric H), 4.78 (1H, dd, J = 3, 1 Hz, anomeric H), 4.83 (1H, dd, J = 9.6, 2 Hz, anomeric H), 4.98 (1H, dd, J = 3.4, 1 Hz, anomeric H), 5.36 (1H, br s, 6-CH), 5.52 (1H, br s, 2'-CH). ^{13}C-NMR (Tables 2 and 3).

Wilfoside C2G (**6**). An amorphous powder, R_{f_3} 0.41, m.p. 135–138°, $[\alpha]_D$ −37.8° (c = 1.00, $CHCl_3$). (Found: C, 59.09; H, 8.19. Calc for $C_{61}H_{98}O_{24} \cdot 3/2H_2O$: C, 58.99; H, 8.12%.) UV $\lambda_{max}^{ethanol}$ nm (log ε): 216 (4.06), 276 (3.66). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1710, 1640, 1170. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 6.7 Hz, 5',6'-CH_3), 1.13 (3H, s, 18-CH_3), 1.41 (3H, s, 19-CH_3), 2.13 (3H, s, 7'-CH_3), 2.17 (3H, s, 21-CH_3), 3.38, 3.42, 3.45 (each 3H, s, 3-OCH_3 of sugar moiety), 4.39 (1H, d, J = 7.9 Hz, anomeric H of β-D-glucopyranose), 4.56 (1H, br t, J = 8.5 Hz, 12-CH), 4.76 (1H, dd, J = 10, 2 Hz, anomeric H), 4.80 (1H, dd, J = 3, 1 Hz, anomeric H), 4.93 (1H, dd, J = 10, 2 Hz, anomeric H), 5.05 (1H, dd, J = 3, 1 Hz, anomeric H), 5.36 (1H, br s, 6-CH), 5.52 (1H, br s, 2'-CH). ^{13}C-NMR (Tables 2 and 3).

Acidic hydrolysis of **6**. A soln of **6** (1 mg) in MeOH (1 ml) was allowed to react with 0.1 N H_2SO_4 (1 ml) at 60° for 15 min, then H_2O (1 ml) was added to this and the whole was concentrated to 2 ml. The soln was kept at 60° for a further 30 min, and neutralized with satd $Ba(OH)_2$. The ppts were filtered off and the filtrate was evaporated to dryness. The products were identified as **7**, **8**, cymarose, **11** and **12** by TLC comparison with the authentic samples. R_f values: **7** (R_{f_2} 0.33, R_{f_6} 0.35), **8** (R_{f_4} 0.56, R_{f_6} 0.35), cymarose (R_{f_4} 0.59, R_{f_6} 0.42), **11** (R_{f_4} 0.42, R_{f_6} 0.04), and **12** (R_{f_5} 0.16, R_{f_7} 0.30).

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